Longitudinal Changes in Pancreatic β-Cell Function and Metabolic Clearance Rate of Insulin in Pregnant Women With Normal and Abnormal Glucose Tolerance

PATRICK M. CATALANO, MD
NOREEN M. DRAGO, BS
SAEID B. AMINI, MBA, PHD

OBJECTIVE — To evaluate basal pancreatic β-cell secretion and suppression during infused insulin and the metabolic clearance rate of insulin in women with normal and abnormal glucose tolerance prior to conception and during pregnancy.

RESEARCH DESIGN AND METHODS — Seven women with normal glucose tolerance and nine women with abnormal glucose tolerance during gestation were evaluated prior to conception, in early (12–14 weeks) and late (34–36 weeks) gestation. Basal insulin and C-peptide were measured after an 11-h fast and during the last 40 min of a 2-h hyperinsulinemic-euglycemic clamp at 40 mU · m^{-2} · min^{-1}. Suppression of basal C-peptide was calculated as the steady-state C-peptide/basal C-peptide. The metabolic clearance rate of insulin was calculated by dividing the insulin infusion rate by the steady-state insulin concentration, which was corrected for residual β-cell secretion.

RESULTS — No significant differences were noted in the following parameters between women with normal and abnormal glucose tolerance with advancing gestation: increase in basal insulin ($P = 0.20$) and C-peptide ($P = 0.12$), ability of infused insulin to decrease basal C-peptide concentration ($P = 0.22$), and metabolic clearance rate of insulin ($P = 0.76$). There was a significant 65% increase in both basal insulin ($P = 0.0005$) and C-peptide ($P = 0.0002$) concentrations in all subjects with advancing gestation. There was a significant ($P = 0.0001$) decrease in the ability of the infused insulin to decrease basal C-peptide concentration. C-peptide as a percentage of the basal was 64% before conception, 74% in early pregnancy, and 108% in late pregnancy. The metabolic clearance rate of insulin significantly ($P = 0.0005$) increased with advancing gestation: pregravid 422 ml · m^{-2} · min^{-1}, early pregnancy 514 ml · m^{-2} · min^{-1}, and 526 ml · m^{-2} · min^{-1} in late pregnancy.

CONCLUSIONS — Pregnancy is accompanied by progressive alterations in insulin kinetics, which are partly responsible for the hyperinsulinemia of this condition. These alterations are more likely a homeostatic response to the increased physiological insulin resistance of pregnancy.

There are significant increases in basal and postprandial insulin concentrations with advancing gestation in gravid as compared with nongravid women (1–3). One possible explanation for the increases in peripheral insulin concentration is β-cell response to decreases in both hepatic and peripheral insulin sensitivity with advancing gestation. However, circulating levels of insulin also represent a balance between the rate of insulin secretion and its metabolic clearance rate.

Under usual conditions, increased plasma insulin concentrations result in decreased glucose concentration, which then results in a decreased secretion of insulin. However, under the conditions of the hyperinsulinemic-euglycemic clamp, infusion of insulin results in direct or indirect negative feedback signals to β-cell secretion of insulin (4). In nongravid individuals, parenterally infused insulin results in a 50% decrease in basal C-peptide concentrations (4). To the best of our knowledge, this has never been longitudinally evaluated during gestation. Additionally, the metabolic clearance rate of insulin during pregnancy has previously been reported as being either increased (5) or not altered when compared with nonpregnant women (6–8). Furthermore, alterations in the metabolic clearance rate of insulin have not been evaluated in the pathogenesis of gestational diabetes. Since previous studies have demonstrated a decrease in insulin response as one of the primary metabolic abnormalities in women developing gestational diabetes (9,10), β-cell function may be an important factor in the pathophysiology of this condition.

Our previous studies (10–12) have reported on the longitudinal changes in insulin response, endogenous glucose production and suppression during insulin infusion, and measurements of insulin sensitivity using the hyperinsulinemic-euglycemic clamp. Therefore, the primary purpose of this aspect of the study was to evaluate the pregravid status and longitudinal changes in *1*) the ability of parenterally infused insulin to inhibit basal pancreatic β-cell production of insulin and 2) the metabolic clearance rate of insulin under steady-state conditions, in women with normal and abnormal glucose tolerance. The null hypotheses to be tested were that *1*) exogenous insulin infusion decreases basal pancreatic β-cell secretion of C-peptide to approximately the same degree both before and during pregnancy, and 2) there is no significant change or difference in the metabolic clearance rate of insulin with advancing gestation, in women with normal or abnormal glucose tolerance.

From the Department of Reproductive Biology, Case Western Reserve University at MetroHealth Medical Center, Cleveland, Ohio.

Address correspondence and reprint requests to Patrick M. Catalano, MD, Department of Reproductive Biology, Case Western Reserve University, MetroHealth Medical Center, 2500 MetroHealth Dr., Cleveland, OH 44109. E-mail: pcatalano@metrohealth.org.

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RESEARCH DESIGN AND METHODS

— This prospective study was performed at the Medical Center Hospital of Vermont between 1986 and 1991. The studies were approved by the hospital's Institutional Review Board, and informed written consent was obtained from each subject before the evaluation. As part of our ongoing studies of changes in prenatal maternal metabolism during pregnancy, we have previously reported on the longitudinal changes in insulin response to infused glucose, basal endogenous glucose production, and suppression during insulin infusion and peripheral insulin sensitivity using the hyperinsulinemic-euglycemic clamp in 15 of the 16 women in this report. A detailed description of the methodologies and results has previously been published (10–12).

Subjects

Sixteen healthy nonobese women were recruited to participate in this study. They were all planning to conceive as soon as the baseline pregravid studies were completed. None of the 16 subjects were breastfeeding, using oral contraceptives, tobacco, or other medications that could affect glucose metabolism. All 16 subjects had normal 75-g oral glucose tolerance tests before conception (13).

Seven women were recruited as control subjects. None had a history of gestational diabetes or complications in a previous pregnancy. Two of these seven women had a family history consistent with NIDDM. All seven women had normal 100-g, 3-h oral glucose tolerance (14) during the index pregnancy and comprised the control group.

Nine women were at high risk of developing gestational diabetes because of a past history of gestational diabetes or an abnormal glucose screening test in a prior pregnancy. Six of these nine women also had a positive family history of NIDDM. Other than gestational diabetes, these pregnancies were uncomplicated. Eight of the nine subjects at risk for gestational diabetes had two or more abnormal plasma glucose values on their 100-g oral glucose tolerance test according to the criteria of Carpenter and Coustan (14). The other subject had one abnormal value on their oral glucose tolerance test. We elected to include the one woman with one abnormal glucose value on their oral glucose tolerance test together with the eight women with gestational diabetes and designate these women as the abnormal glucose tolerance group. Recent studies (15,16) have shown that one abnormal value on the 3-h glucose tolerance test is strongly associated with adverse perinatal outcome related to gestational diabetes. Additionally, there were no significant differences in the results when the data were analyzed using either the nine women with abnormal glucose tolerance or eight women with gestational diabetes in comparison to the control group.

Experimental protocol

Each subject was evaluated before conception, in early pregnancy (12–14 weeks gestation), and again in late pregnancy (34–36 weeks gestation). Each subject was instructed in a standard diet 2 weeks before each study by the General Clinical Research Center nutritionist to standardize nutrition intake between groups. The composition of the diet was identical to that used in the treatment of gestational diabetes at our institution.

At the time of diagnosis of abnormal glucose tolerance, all subjects were taught to perform home blood glucose monitoring with either a reflectance meter or visual inspection of glucose reagent strips. Target fasting and preprandial glucose values were <100 mg/dl and postprandial values were <120 mg/dl. Two subjects eventually required insulin therapy because they exceeded target glucose values on diet alone. Physical activity was maintained throughout gestation.

A detailed description of the specific methodology used in our study protocol has been published previously (10). Briefly, screening for islet cell antibodies was performed using a specific monoclonal antibody at the Joslin Diabetes Center (17). Body composition was estimated by hydrodensitometry with correction for residual lung volume by helium dilution (18). The percent body fat was calculated according to Keys and Brozek (19) to estimate fat-free mass. The decrease in basal C-peptide concentration during parenterally infused insulin and the metabolic clearance rate of insulin under steady-state conditions were performed during the hyperinsulinemic-euglycemic clamp. The hyperinsulinemic-euglycemic clamp was performed as described by DeFronzo et al. (20) and as described earlier (10). Blood samples were obtained in heparinized syringes. The blood samples were immediately centrifuged and an aliquot was assayed for glucose by the glucose oxidase method using a Yellow Springs glucose analyzer (Yellow Springs, OH). The remainder of the plasma sample was placed in tubes without preservative for insulin assay. Blood samples were collected and placed in prechilled tubes containing 100 μU of Trasylol/ml of whole blood (Miles, Kankakee, IL) for measurement of plasma C-peptide, spun and frozen at −80° C for later analysis. Insulin and C-peptide samples were batched so that each subject's pregravid, early and late pregnancy samples would be analyzed together to decrease interassay variation in results. All radioimmunoassays were performed in duplicate. Insulin assays were performed using a double antibody technique according to the method of Starr et al. (21). The interassay coefficient of variation was 7.1%, and intraassay coefficient of variation was 6.0%. C-peptide was measured according to the method of Starr et al. (22). The interassay coefficient of variation was 7.2% and the intraassay coefficient of variation was 3.5%.

C-peptide as a percentage of the basal level was calculated as the mean steady-state C-peptide concentration during the clamp divided by the average basal C-peptide concentration.

The metabolic clearance rate of insulin was calculated (modification from Minaker et al. [23]), as the infusion rate of insulin during steady state divided by the steady-state insulin concentration multiplied by fasting or basal insulin corrected for suppression of basal C-peptide during the clamp.

$$MCR = \frac{I}{SSI \times FI \times \left(\frac{SS\ C\text{-}pep}{F\ C\text{-}pep}\right)}$$

where *MCR* is metabolic clearance rate of insulin (milliliter per square meter per minute); *I* is insulin infusion rate (picomole per square meter per minute) during the clamp; *SSI* is insulin concentration during steady-state infusion during the clamp (picomole per liter); *FI* is fasting or basal insulin (picomole per liter); *SS C-pep* is steady-state C-peptide during the clamp (nanomole per liter); and *F C-pep* is fasting C-peptide (nanomole per liter).

Basal or fasting insulin was corrected for the *SS C-pep/F C-pep* because infused insulin was unable to completely suppress the basal insulin production and the inability of our insulin assay to distinguish infused from endogenous insulin.

Statistical analysis

The data are expressed as mean ± SD. Statistical analyses were performed using

Table 1—*Demographic characteristics of study subjects*

	Normal glucose tolerance	Abnormal glucose tolerance	*P* value
n	7	9	—
Age (years)	31 ± 5.5	31.7 ± 2.6	0.63
Parity (%)			
0–1	6 (76)	8 (89)	1.0
>1	1 (14)	1 (11)	
Weight (kg)	57.0 ± 7.8	58.9 ± 6.0	0.79
Height (cm)	166.4 ± 6.4	168.9 ± 8.3	0.56
BMI (kg/m^2)	20.5 ± 2.1	20.6 ± 1.2	0.87
Fat mass (kg)	10.1 ± 3.6	12.9 ± 2.5	0.12
Body fat (%)	17.5 ± 5.2	21.8 ± 3.3	0.07
Lean body mass (kg)	46.8 ± 5.8	46.0 ± 4.8	0.26
Months to conception	3.0 ± 2.5	3.8 ± 3.3	0.99

Data are means ± SD or *n* (%).

Mann-Whitney's *U* test, Fisher's exact test, analysis of variance with repeated measures for two groups, and linear regression analysis. The repetitive analysis of variance was used to evaluate the longitudinal changes in a given parameter over time, between groups, and group-by-time interactions. Statistical analysis was performed using the SAS Software (Cary, NC). $P \leq 0.05$ was considered significant.

RESULTS — The pregravid demographics of our study population are given in Table 1. There were no significant differences in any of the demographic characteristics between the women with normal and abnormal glucose tolerance. Islet cell antibody measurements were negative in all study subjects.

There was a significant ($P = 0.0005$) increase in basal C-peptide and basal insulin concentration ($P = 0.0002$) from the time before conception through late gestation in our study subjects. There were, however, no significant differences in either basal insulin ($P = 0.20$) (10) or C-peptide concentrations ($P = 0.12$) between women with normal versus abnormal glucose tolerance (Table 2). Additionally, there was no significant correlation between basal C-peptide and either basal hepatic glucose production or insulin sensitivity before conception in early or late gestation (data not shown).

The results of the C-peptide and insulin concentrations obtained during the hyperinsulinemic-euglycemic clamp are shown on Table 3. There was a significant increase in C-peptide concentration ($P = 0.0002$) with advancing gestation but no significant difference ($P = 0.60$) between subjects with normal as compared with abnormal glucose tolerance. In contrast, there was a significant decrease in insulin concentration obtained during the clamp ($P = 0.004$), particularly from the time before conception to early pregnancy (618 vs. 498 pmol/l). However, mean insulin concentration during the clamp increased in late gestation (564 pmol/l). There were again no significant differences ($P = 0.69$) in clamp insulin concentrations between study groups.

There was a significant ($P = 0.0001$) decrease in the ability of the infused insulin to decrease basal C-peptide concentrations (Fig. 1). By late gestation, mean C-peptide concentrations during the clamp were generally equivalent to basal levels. C-peptide as a percentage of the basal level in the women with normal glucose tolerance was 74 ± 33% before conception, 93 ± 55% in early pregnancy, and 113 ± 40% in late pregnancy. In women with abnormal glucose tolerance, C-peptide as a percentage of basal level was 57 ± 18% before conception, 60 ± 18% in early pregnancy, and 104 ± 40% in late pregnancy. There was no significant ($P = 0.22$) difference between groups.

The metabolic clearance rate of insulin over time in women with normal and abnormal glucose tolerance is shown in Fig. 2. Before conception, the metabolic clearance rate of insulin was 440 ± 73 ml · m^{-2} · min^{-1} in subjects with normal glucose tolerance and 410 ± 90 ml · m^{-2} · min^{-1} in women with abnormal glucose tolerance. However, with advancing gestation there was a significant ($P = 0.0005$) increase, but no difference ($P = 0.77$) in the metabolic clearance rate of insulin in women with normal and abnormal glucose tolerance. Based on these data, 40 subjects in each group would be required to show a significant difference (at the 0.05 level) in insulin clearance between the two groups during pregnancy. There was no significant correlation between the metabolic clearance rate of insulin and maternal weight, fat-free mass, percent body fat, insulin sensitivity, or placental weight at any time during the period of study (data not shown). The only significant correlation of the metabolic clearance rate of insulin was with creatinine clearance before conception ($r = 0.64$, $P = 0.01$) but not at any time during gestation.

CONCLUSIONS — The results of our study confirm previous reports describing a significant increase in basal insulin concentrations with advancing gestation (1–3). The increase in basal C-peptide concentration during gestation is consistent with the increase in basal insulin. We speculate that the increases in basal insulin and C-peptide are in response to increased basal endogenous (primarily hepatic) insulin resistance with advancing gestation (10). Although basal insulin and C-peptide concentrations were greater in the lean women with abnormal as compared with lean women with normal glucose tolerance, in late gestation,

Table 2—*Longitudinal change in basal insulin and C-peptide concentration*

	n	Pregravid	Early gestation	Late gestation
Basal insulin (pmol/l)				
Normal glucose tolerance	7	35 ± 18	28 ± 11	70 ± 29*
Abnormal glucose tolerance	9	55 ± 28	34 ± 10	80 ± 32*
Basal C-peptide (nmol/l)				
Normal glucose tolerance	7	0.37 ± 0.27	0.32 ± 0.14	0.70 ± 0.34†
Abnormal glucose tolerance	9	0.60 ± 0.30	0.50 ± 0.18	0.90 ± 0.45†

Data are means ± SD. Change over time: *$P = 0.0002$; †$P = 0.0005$.

Table 3—*Insulin and C-peptide concentrations during the hyperinsulinemic-euglycemic clamp*

	n	Pregravid	Early gestation	Late gestation
Insulin (pmol/l)				
Normal glucose tolerance	7	582 ± 103	479 ± 80	588 ± 151*
Abnormal glucose tolerance	9	643 ± 113	515 ± 79	544 ± 106*
C-peptide (nmol/l)				
Normal glucose tolerance	7	0.24 ± 0.18	0.27 ± 0.14	0.83 ± 0.52†
Abnormal glucose tolerance	9	0.32 ± 0.15	0.30 ± 0.15	0.91 ± 0.49†

Data are means ± SD. Change over time: *P_t = 0.004; †P_t = 0.0002.

125 subjects would need to be evaluated to have a power of 0.80 to show a significant difference (0.05) between basal insulin concentrations (67 for basal C-peptide). These results may not apply to obese women with normal as compared with abnormal glucose tolerance. Additionally, the suppression of pregravid basal C-peptide concentration during insulin infusion during the hyperinsulinemic-euglycemic clamp is comparable to the decrease in nonpregnant lean control subjects as reported by Elahi et al. (4) using similar methodology.

In contrast, during gestation, however, the basal C-peptide did not decrease to the same degree or even to a similar percentage decrease with insulin infusion during the hyperinsulinemic-euglycemic clamp as compared with pregravid measurements. These results are in contrast to the results of Elahi et al. (4) in nongravid subjects. Whereas obese Caucasian subjects and obese Pima Indians had elevated basal C-peptide concentrations in comparison with lean Caucasian subjects, the absolute suppression of C-peptide was greater, and the percentage suppression was the same as lean Caucasian subjects. Although a decrease in the metabolic clearance rate of C-peptide during gestation might possibly account for the increase in basal concentration and decrease in suppression of basal C-peptide during insulin infusion with advancing gestation, we believe this not to be the case. We speculate that these results may represent another effect of the hormonal milieu of pregnancy on maternal metabolism.

The metabolic clearance rate of C-peptide is not altered in nonpregnant animal studies when its concentrations are increased from 0.28 to 1.45 pmol/nl with intravenous infusion (24). Additionally, the mean 65% increases in basal C-peptide concentration observed during gestation would be difficult to reconcile with a basal hepatic extraction of only 3–4% as described by Polonsky (25) in nonpregnant subjects. We speculate that the increases in basal C-peptide and decreases in suppression during insulin infusion with advancing gestation represent either an increase in β-cell response to decreases in hepatic and/or peripheral insulin sensitivity or possibly a direct effect of the pregnancy hormonal milieu on β-cell function.

There is evidence in the pregnant rat model for both increases in maternal islet cell size and insulin secretion during gestation. Hellerman (26) described a significant increase in pancreatic islet cell size in pregnant rats at delivery as compared with nonpregnant rats. In a separate report, Kalkhoff (27) reported that pregnant rats secrete significantly more insulin per unit islet cell mass as compared with virgin rats using an in vitro islet cell preparation. However, the signals for these alterations in β-cell function during gestation have not been well described. Although there was only a marginally significant correlation (r = 0.50, P = 0.048) between suppression of basal C-peptide during insulin infusion and insulin sensitivity in early gestation, β-cell response to decreases in insulin sensitivity may be a contributing factor in early pregnancy. Likewise, a direct effect of the various placental hormones of pregnancy on maternal β-cell function was not examined. Increases in insulin response during the intravenous glucose tolerance test in early gestation in subjects with abnormal glucose tolerance and no change in either basal hepatic glucose turnover or peripheral insulin sensitivity (10), however, lends indirect support to this hypothesis.

Previous investigators have reported either no change or increase in the metabolic clearance rate of insulin during gestation. In separate studies, Bellman (6), Lind (7), and Burt (8) have reported no difference in the insulin disappearance curves/rate when insulin was infused intravenously at various concentrations to pregnant women in late gestation and in nonpregnant subjects. In contrast, Goodner and Freinkel (5), using an I^{131}-labeled insulin infusion, described a 25% increase in insulin turnover in the pregnant as compared with a nonpregnant rat model. Our results support those of Goodner and Freinkel (5), whereby we observed a mean 24% increase in the metabolic clearance rate of insulin in our study subjects. We speculate that in the studies showing no change in the metabolic clearance rate of insulin during gestation, the methods used to measure infused immunoreactive insulin were not able to distinguish between the infused exogenous

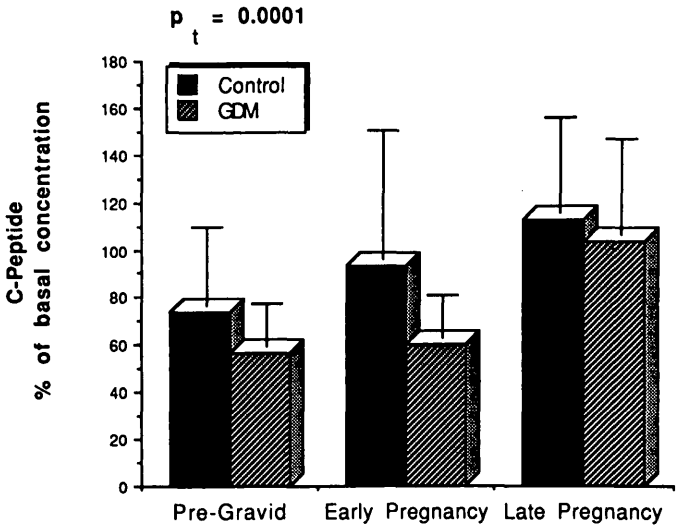

Figure 1—*Longitudinal changes (means ± SD) in basal C-peptide vs. percentage of basal concentration during infusion of insulin and glucose during the hyperinsulinemic-euglycemic clamp.* P_t = *change over time.*

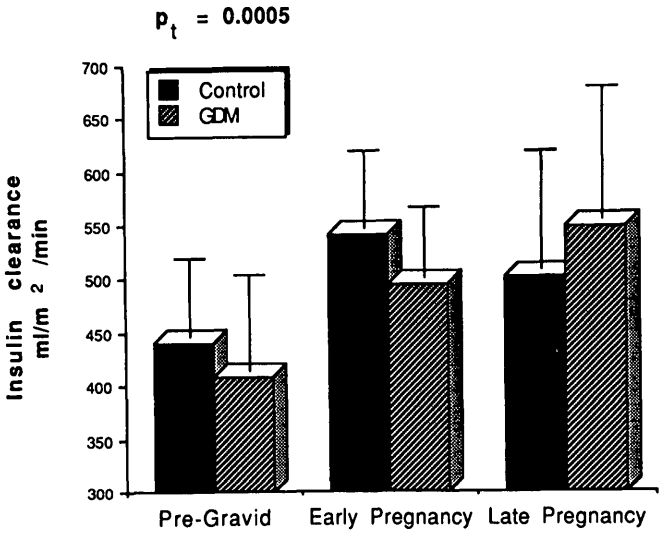

Figure 2—*Longitudinal change (means ± SD) in insulin clearance during the hyperinsulinemic-euglycemic clamp.* P_t = *change over time.*

insulin and the increases in basal endogenous insulin production plus impaired suppression of insulin secretion by glucose and insulin. This may have resulted in there being no "net" change in the insulin degradation curves in late pregnancy as compared with the nonpregnant subjects. A similar mechanism may explain the decrease in insulin concentration during the clamp in early as compared with either pregravid or late gestation. Relative to pregravid measurements, there was 10% less suppression of basal C-peptide but a 22% increase in the metabolic clearance rate of insulin during insulin infusion in early gestation. This may have resulted in a decrease in mean insulin concentration (618 vs. 498 pmol/l) achieved during the clamp. In contrast (relative to pregravid measurements) there was no suppression but rather an 8% increase in C-peptide concentration during the clamp relative to basal measurements and a 24% increase in the metabolic clearance rate of insulin during insulin infusion in late gestation. Hence, these alterations in insulin kinetics may have resulted in the increased mean insulin concentrations achieved during the clamp in late as compared with early pregnancy (564 vs. 498 pmol/l).

The mean pregravid estimates of the metabolic clearance rate of insulin in our nonobese subjects (422 ml · m^{-2} · min^{-1}) was similar to that reported by Elahi et al. (4) (439 ml · m^{-2} · min^{-1}). The organs primarily responsible for insulin clearance in nonpregnant individuals are liver, kidney, and muscle (28). Approximately 50% of insulin is extracted during the first pass through the liver (29). Interestingly, the only significant correlation we observed was between pregravid insulin clearance and creatinine clearance, thereby supporting the role of the kidney in insulin clearance in our study subjects. Insulin clearance is reported to be decreased in the elderly (23) and in conditions associated with increased insulin resistance, e.g., obesity (30). Under experimental conditions, insulin clearance is saturable when insulin levels of ~200 μU/ml are reached (23). Although we found no correlation between placental weight and insulin clearance in late gestation, we speculate that the placenta accounts for the increase in insulin clearance with gestation. As described by Goodner and Freinkel (31) and later by Posner (32), the placenta is rich in enzymes capable of degrading insulin.

On the basis of our results, we were unable to disprove our null hypothesis that there was a significant difference in the ability of infused insulin to decrease β-cell secretion of C-peptide and metabolic clearance rate of insulin in lean women with normal and abnormal glucose tolerance during pregnancy. We speculate that the metabolic stress of pregnancy on maternal pancreatic β-cell function was similar for lean women with normal and abnormal glucose tolerance. Whether these differences between groups apply to obese women remains speculative, but we are currently in the process of assessing this and other aspects of maternal glucose metabolism in obese women with normal and abnormal glucose tolerance.

In summary, we observed a significant increase in basal insulin and C-peptide from the time before conception through late gestation, which did not suppress during exogenous insulin infusion. There was a significant increase in the metabolic clearance rate of insulin with advancing gestation. This increase in the metabolic clearance rate of insulin during pregnancy, however, did not correlate with maternal weight, fat-free mass, percent body fat, insulin sensitivity, creatinine clearance, or placental weight. Lastly, there were no significant differences in the ability of exogenous insulin to suppress basal C-peptide secretion or the metabolic clearance rate of insulin in women with normal as compared with abnormal glucose tolerance. These data support the unique role of pregnancy on maternal carbohydrate metabolism.

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